sequence, with the principal difference located at 23 C-terminal residues spanning the hypervariable region (HVR) of the protein. The primary objective of this investigation is to understand, on a molecular and functional level, why different abundances of the K-Ras isoforms 4A and 4B are present in different cancers. Recent molecular dynamics (MD) and NMR studies on the K-Ras4B isoform suggest a novel interaction of the HVR with the catalytic domain of the protein that may confer auto-regulatory Ras activity. Here, we examine whether the K-Ras4A isoform exhibits mechanistic differences that may contribute to its differences in oncogenic signaling. To accomplish this aim, specific actions currently being undertaken include (1) MD studies of the K-Ras4A catalytic domain, (2) characterization of the K-Ras4A HVR structure and its conformational fluctuations, (3) MD studies of the full-length K-Ras4A isoform, and (4) experimentally-informed characterization of potential HVR interactions with the catalytic domain of the protein. Elucidating the functional significance of the K-Ras4A HVR and determining whether it is able to modulate Ras catalytic activity would have important implications towards the development of medicinal approaches to cancer treatment, and enhance current knowledge about cellular oncogenic signal transduction pathways. Funding: National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E.

1056-Pos Board B7

Theoretical Study of the Protein Folding Dynamics from a Time Correlation Function Approach

Toshifumi Mori, Shinji Saito.
Institute for Molecular Science, Okazaki, Japan.

With the development of experimental approaches such as single molecule experiments and the advances in computer simulation techniques (including the emerge of the specialized hardware Anton), the knowledge of protein folding mechanism, especially in the microsecond regime, has greatly deepened over the last few decades. While the sub-millisecond to millisecond long simulations at atomic detail can be extremely insightful, extracting the events mined in the high dimensional trajectory and understanding the dynamics in detail is still a challenging issue; especially how to interpret such high dimensional, long time-series data is not well established.

We propose a protocol to extract the ''slow'' modes directly from the trajectory with the use of the time correlation functions in the high dimensional space. We apply the current approach to a previously reported 100 μs trajectory of the FiP35 WW domain (Science 330 (2010), 341-346). We show that the slowest mode extracted successfully describes the folding/unfolding event with the time scale of ~8 μs, consistent with previous studies. In addition, we find that the formation of only the longer β-sheet seems to be the dominant intermediate state along the folding pathway, and furthermore identify a misfolded state which lasts up to a few μs. The time scale of the latter two events are found to be a few hundred ns, respectively. These results show that by using the time-series information, the dynamics of protein folding can be understood in greater detail.

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Extensive Conformational Heterogeneity within Protein Cores

Gregory R. Bowman.
Biochemistry & Molecular Biophysics, Washington University School of Medicine, St Louis, MO, USA.

Basic principles of statistical mechanics require that proteins sample an ensemble of conformations at any nonzero temperature. However, it is still common to treat the crystallographic structure of a protein as the single structure of its native state, largely because high-resolution structural characterization of protein flexibility remains a profound challenge. To assess the typical degree of conformational heterogeneity within folded proteins, we construct Markov state models describing the thermodynamics and kinetics of proteins ranging from 72 to 263 residues in length. Each of these models is built from hundreds of microseconds of atomically detailed molecular dynamics simulations. Examination of the side-chain degrees of freedom reveals that almost every residue visits at least two rotameric states over this time frame, with rotamer transition rates spanning a wide range of time scales (from nanoseconds to tens of microseconds). We also report substantial backbone dynamics on time scales longer than are typically addressed by experimental measures of protein flexibility, such as NMR order parameters. Finally, we demonstrate that these extensive rearrangements are consistent with room-temperature crystallographic data and NMR experiments probing both ps-ns and μs-ms timescales. Altogether, these results depict the interior of proteins not as well-ordered solids, as is often imagined, but instead as dense fluids, which undergo substantial structural fluctuations despite their high packing fraction. Moreover, the degree of agreement between our models and experiments suggests the ensembles we generate are an accurate representation of reality and should be of tremendous value for understanding and rationally manipulating biomolecules.

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Conformational Dynamics of the Pili Constructing Sortase C Enzymes

Emmanuel B. Naziga[1], Jeff Wereszczynski[2].
[1]Illinios Institute of Technology, Chicago, IL, USA, [2]Physics, Illinios Institute of Technology, Chicago, IL, USA.

Pili are elongated protein structures that enhance the adhesive abilities and virulence of bacteria. Therefore, understanding the pilin assembly process has important ramifications for the development of new antibacterial compounds. In streptococcus pnuenomae, three Sortase C (SrtC) enzymes are responsible for the construction of pili. In contrast to the housekeeping SrtA class of proteins, SrtC enzymes possess a flexible lid that is thought to modulate access to the active site during the pili construction process. In this work, we have used molecular dynamics simulations, including advanced sampling techniques, to study the conformational dynamics of SrtC. Here, we have probed the lid opening mechanism of three SrtC enzymes (SrtC-1, SrtC-3 and SrtC-3) as well as for two experimentally studied mutants of SrtC-1 (K58G/P60G and R202E). The results suggest that the aforementioned mutations lead to an open lid conformation in SrtC-1, in accordance with experimental findings. In contrast, the salt bridge between the conserved aspartate residue in the lid region and arginine in the active site leads to a robust anchoring of the lid that is highly stable during long conventional molecular dynamics simulations of all the SrtC proteins. This finding is confirmed by umbrella sampling free energy calculations. Excursions from this anchored position are very brief and are to a conformation with a bridged hydrogen bond between the two residues that is mediated by a water molecule. Overall, results show that opening of the active site of SrtC to initiate pili assembly likely requires interactions that are provided by the sorting signal that is contained in the pilin subunit building blocks.

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Targeted Conformational Transitions of Multimeric Proteins by Monte Carlo Simulations Combined with Collective Anisotropic Network Model Modes

Yasemin Yesiltepe[1], Arzu Uyar[1], Deniz Turgut[2], Turkan Haliloglu[1], Pemra Doruker[1], **Rahmi Ozisik**[2].
[1]Chemical Engineering, Bogazici University, Istanbul, Turkey, [2]Materials Science & Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA.

Monte Carlo simulations combined with anisotropic network model (ANM-MC) were used to investigate transition pathways between two different crystal structures such as open and closed states of proteins. The transition pathway is identified by an iterative process that involves selecting the collective ANM mode that overlaps best with the target direction and minimizing the conformational energy of the selected ANM mode via Monte Carlo simulations. In the current study, the conformational transition pathways and pathway intermediates involving shear and hinge type of motions were analyzed via the ANM-MC algorithm. The results show that the collective modes contribute significantly to the conformational transitions in both types of motions. In hinge-bending proteins, generally a single low-indexed mode predominantly drives the conformational transition, whereas a collection of slow modes is necessary to describe a shear-type transition. Targeted ANM-MC approach was found to provide and efficient method to explore sequences of events in transition pathways in both types of motions. Applications of the algorithm to large, multi-chain proteins will also be presented. A computer server based on the ANM-MC algorithm will be made available to the scientific community.

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Conformational Fluctuations as an Intrinsic Mechanism of Action of Lipase Foldase Studied by the High-Precision FRET Toolkit and MD Simulations

Jakub Kubiak[1], Filip Kovacic[2], Peter Dollinger[2], Florian Bleffert[2], Karl-Erich Jaeger[2], Holger Gohlke[3], Claus A.M. Seidel[1].
[1]Institute for Molecular Physical Chemistry, Universität Düsseldorf, Düsseldorf, Germany, [2]Institute for Molecular Enzyme Technology, Universität Düsseldorf, Jülich, Germany, [3]Institute for Pharmaceutical and Medicinal Chemistry, Universität Düsseldorf, Düsseldorf, Germany.

The lipase foldase (Lif) of P. aeruginosa is a steric chaperon, which acts as a conformational switch for the activation of lipase A (LipA). In the Lif:LipA complex, Lif forms flexible α-helical scaffold embracing LipA in headphone-like structure. In the unbound form, Lif does not stay in the hollow ''headphone'' conformation but rather exhibits large-scale conformational dynamics, where this α-helical structure undergoes reversible collapses and extensions on the microsecond to sub-microsecond timescale. This process